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Mood Stabilizers Target Cellular Plasticity and Resilience Cascades

Implications for the Development of Novel Therapeutics

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Abstract

Bipolar disorder is a devastating disease with a lifetime incidence of about 1% in the general population. Suicide is the cause of death in 10 to 15% of patients and in addition to suicide, mood disorders are associated with many other harmful health effects. Mood stabilizers are medications used to treat bipolar disorder. In addition to their therapeutic effects for the treatment of acute manic episodes, mood stabilizers are useful as prophylaxis against future episodes and as adjunctive antidepressant medications. The most established and investigated mood-stabilizing drugs are lithium and valproate but other anticonvulsants (such as carbamazepine and lamotrigine) and antipsychotics are also considered as mood stabilizers. Despite the efficacy of these diverse medications, their mechanisms of action remain, to a great extent, unknown. Lithium's inhibition of some enzymes, such as inositol monophosphatase and glycogen synthase kinase-3, probably results in its mood-stabilizing effects. Valproate may share its anticonvulsant target with its mood-stabilizing target or may act through other mechanisms. It has been shown that lithium, valproate, and/or carbamazepine regulate numerous factors involved in cell survival pathways, including cyclic adenine monophospate response element-binding protein, brain-derived neurotrophic factor, bcl-2, and mitogen-activated protein kinases. These drugs have been suggested to have neurotrophic and neuroprotective properties that ameliorate impairments of cellular plasticity and resilience underlying the pathophysiology of mood disorders. This article also discusses approaches to develop novel treatments specifically for bipolar disorder.

Index Entries: Mania; depression; manic-depressive illness; lithium; valproate; neurotrophic factors; pharmacology, bcl-2; neuroplasticity; mitochondria.

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Introduction

Bipolar disorder is a devastating and relatively common disease, with an overall lifetime incidence of about 1% in the general population. Numerous studies have shown that the outcome is poor for a high percentage of patients, with a high rate of chronicity, residual symptoms, relapse, subsyndromes, cognitive and functional impairment, and psychosocial disability *(1–6)*. The cost associated with disability and premature death represents an economic burden of tens of billions of dollars annually in the United States. Not surprisingly, the Global Burden of Disease Study identified bipolar disorder and mood disorders as one of the leading causes of disability worldwide, with increasing disability likely in the coming years *(7)*. In addition to the tremendous economic cost, suicide is estimated to be the cause of death in 10 to 15% of the individuals with bipolar disorder; in addition to suicide, mood disorders are associated with many other health-related consequences *(8–10)*.

More than 50 yr ago, the discovery of the efficacy of lithium as an antimanic agent revolutionized the treatment of patients with bipolar disorder. The remarkable efficacy of lithium for both the acute and prophylactic treatment of mania and depression has served to reshape not only medical and scientific but also popular concepts of severe mental illnesses. Efforts to understand how a simple monovalent cation like lithium can exert such profound beneficial effects has led investigators to examine the potential role of signal transduction pathways in bipolar disorder. After more than 50 yr, lithium remains one of the mainstays of treatment for bipolar disorder, and understanding the target of this drug (which generally has clinical specificity) for bipolar disorder still guides many current research efforts.

In the past decade, there has been an increase in the number of options available for the treatment of recurrent mood disorders, with a parallel and unprecedented increase in the interest in the treatment of bipolar disorder by pharmaceutical companies, clinicians, researchers, and the general public. Several newer pharmacological agents are rapidly being introduced into the market and are being used by clinicians for the management of patients with bipolar disorder. However, overall, these are agents that have been developed for other disorders (most notably epilepsy or schizophrenia) that are being investigated for their potential utility in bipolar disorder. Most notable among the anticonvulsant class are the efficacies of valproate, lamotrigine, and carbamazepine. When tested, all antipsychotics (both older generation "typicals" and newer generation "atypicals") have generally been found efficacious for both acute treatment and prophylaxis of mania. Although many of these medications do have some degree of efficacy for treatment of bipolar disorder, they are probably not optimized for its treatment. Therefore, there is a clear need to develop novel therapeutics specifically for bipolar disorder, an approach which is outlined in this article.

Broadly speaking, approaches to develop novel therapeutics for a complex disorder (in which little is *definitely* known regarding the underlying neurobiology) like bipolar illness can take one of three forms: (a) understanding the *initial* biochemical targets of medications currently in use and using that knowledge to design new drugs directed at that target(s); (b) understanding the delayed, presumed most therapeutically relevant biochemical targets of medications currently in use and using that knowledge to design new drugs directed at that target(s); and (c) understanding the pathophysiology of the illness and using that knowledge to design therapeutics to attenuate or prevent that pathological process.

Regarding the first two strategies, it is noteworthy that the most common medications used to treat bipolar disorder (lithium, anticonvulsants, antipsychotics) differ greatly in their structure and in their presumed initial molecular/cellular targets. Valproate is an eight-carbon-branched fatty acid; lithium is a monovalent cation; carbamazepine has a similar structure as the tricyclic antidepressants; and antipsychotics differ widely in structure. With the exception of antipsychotics, these drugs do not

appear to specifically target cell surface receptors *(11,12)* but exert their action on intracellular targets leading to direct and indirect effects on signaling pathways. This direct and indirect regulation of signal transduction pathways by mood stabilizers has clear implications for the delayed onset of their therapeutic effect (i.e., often many weeks, thus likely representing upstream modulators of gene expression, protein levels, and plasticity of neurons, glia, and their synapses) *(13)*.

Because of their shared clinical effect in treating bipolar disorder, investigators are attempting to identify common targets to explain how these different drugs participate in similar pathways and long-term physiological actions *(13–20)*. We also believe that understanding of the critical signaling pathways underlying treatment response will help to unravel the complex pathophysiology of bipolar disorder. This article discusses initial targets of mood-stabilizing drugs (focusing primarily on lithium and valproate), downstream targets shared by multiple drugs, and physiological actions (such as neurotrophic effects) implicated in treatment response. The final section discusses the development of novel drugs.

Primary Direct Targets of Mood-Stabilizing Drugs

Originally, the main focus in the research of the action of mood-stablizing drugs was to understand drug–receptor interactions; however, it has become clear that drugs like lithium and valproate do not appear to have major direct interactions with cell surface receptors and likely exert their effects directly or indirectly through modulation of intracellular targets.

Lithium functions as a direct inhibitor of a limited number of lithium-sensitive enzymes, where it acts as a competitor for magnesium because of their similarly sized ionic radius *(21–23)*. The enzymes that are known to be significantly inhibited by therapeutic serum concentrations (0.6–1.2 m*M*) of lithium are four phosphomonoesterases *(24,25)*: inositol-monophosphatase (IMPase), inositol-polyphosphate 1-phosphatase (IPPase), bisphosphate 3′-nucleotidase, and fructose 1,6-bisphosphatase. Two other enzymes that are direct targets of lithium are glycogen synthase kinase-3 (GSK-3) and phosphoglucomutase *(26,27)*. IMPase and GSK-3 are the direct targets that have received the greatest amount of interest. Therefore, these two enzymes and their respective pathways—namely, the phosphoinositol signaling cascade for IMPase and the Wnt-signaling pathway for GSK-3—are discussed in more detail in the following sections.

Valproate demonstrates clear effects on high-frequency sodium channel firing and γ-aminobutyric acid (GABA)—actions that are likely critically important for its anticonvulsant effects. Known direct targets of valproate are sodium channels, enzymes involved in the metabolism of GABA (GABA-transaminase [GABA-T], succinate semialdehyde dehydrogenase [SSA-DH], succinate semialdehyde reductase), and histone deacetylase (HDAC). Several studies have shown that valproate (at therapeutic concentrations) is an inhibitor of SSA-DH *(28–32)*. This enzyme is critical for the GABA shunt, a pathway that produces both glutamate and GABA by circumventing a portion of the tricarboxylic acid cycle. GABA-T converts GABA to SSA, which is then converted to succinate by SSA-DH. Additionally, valproate inhibits SSA reductase (SSA-R), the enzyme that converts SSA to γ-hydroxybutyrate.

Histones are components of nucleosomes, on which DNA is bound to form chromatin. Acetylation of histones reduces their affinity for DNA and is a major epigenetic regulator of gene expression. Acetylation of histones is regulated by histone acetyltransferases and HDACs and generally activates gene transcription. Two classes of HDACs (classes I and II) are found in large protein complexes that suppress gene transcription (of certain genes in specific cell types). Valproate is an inhibitor of class I HDACs in vitro, in cell culture, and in intact animals with inhibition within therapeutic serum levels (0.4–0.8 m*M*) *(33)*. Recent studies

have found that peripheral valproate administration increases histone acetylation in the rodent brain following short- *(34,35)* and long-term administration *(35)*. The inhibition of HDAC by valproate promotes acetylation and, therefore, has the potential to increase transcription of certain genes. Therefore, the effect of valproate on HDAC may explain many of its effects on cellular signaling pathways.

Signaling Pathways Involved in the Action of Mood Stabilizers

Signaling Networks: The Cellular Machinery Underlying Information Processing and Long-Term Neuroplastic Events

It is hardly surprising that signaling pathways appear to represent core, fundamental targets for the actions of mood stabilizers. Signal transduction pathways are in a pivotal position in the central nervous system (CNS) because they can affect the functional balance between multiple neurotransmitter systems and, therefore, may play a role in mediating the more "downstream" abnormalities that likely underlie the pathophysiology of bipolar disorder. Multicomponent, cellular signaling pathways interact at various levels, thereby forming complex signaling networks that allow the cell to receive, process, and respond to information *(37,38)*. These networks facilitate the integration of signals across multiple time scales as well as the generation of distinct outputs depending on input strength and duration and regulate intricate feedforward and feedback loops *(39)*. These properties of signaling networks suggest they play critical roles in cellular memory; therefore, cells with different histories (which subsequently express a different repertoire of signaling molecules and interact at different levels) may respond quite differently to the same signal over time.

In the CNS, signaling cascades serve the critical roles of first amplifying and "weighting" numerous extracellularly generated neuronal signals and then transmitting these integrated signals to effectors, thereby forming the basis for a complex information processing network *(37,40)*. The high degree of complexity generated by these signaling networks may be one mechanism by which neurons acquire the flexibility for generating the wide range of responses observed in the nervous system. Consequently, these pathways are likely involved in regulating diverse vegetative functions such as mood, appetite, and wakefulness and are likely to be involved in the pathophysiology and treatment of bipolar disorder. The information presented in the next subsections reviews signaling pathways that have received the greatest attention vis-à-vis the clinically relevant actions of mood stabilizers.

Adenylate Cyclase Signaling Pathway/Phosphoinositol Signaling Pathway and Protein Kinase C

Presynaptically released neurotransmitters binding to postsynaptic extracellular membrane receptors activate signal transduction events inside the cell. This signal transduction often is mediated through activation of G proteins consisting of three G protein-coupled receptor subunits (α, β, γ; for a more detailed discussion, *see* ref. *41*).

G protein activation subsequently activates effectors such as adenylate cyclase (AC; Fig. 1A) or phospholipase C (PLC; Fig. 1E) or even ion channels. AC converts adenine triphosphate (ATP) to a second messenger called cyclic adeninine monophosphate (cAMP), which activates protein kinase A (PKA; Fig. 1A). PKA regulates many proteins through phosphorylation, including other enzymes, transcription factors (such as cAMP response element-binding protein [CREB]), ion channels, and cytoskeletal proteins. The literature concerning lithium-induced changes in G protein-coupled receptors and/or G protein subunits describes heterogenous findings (for review, *see* ref. *42*). Overall, the results suggest that basal cAMP levels are increased, whereas stimulus-induced increases in cAMP are blunted. These seemingly opposing effects can be conceptualized as

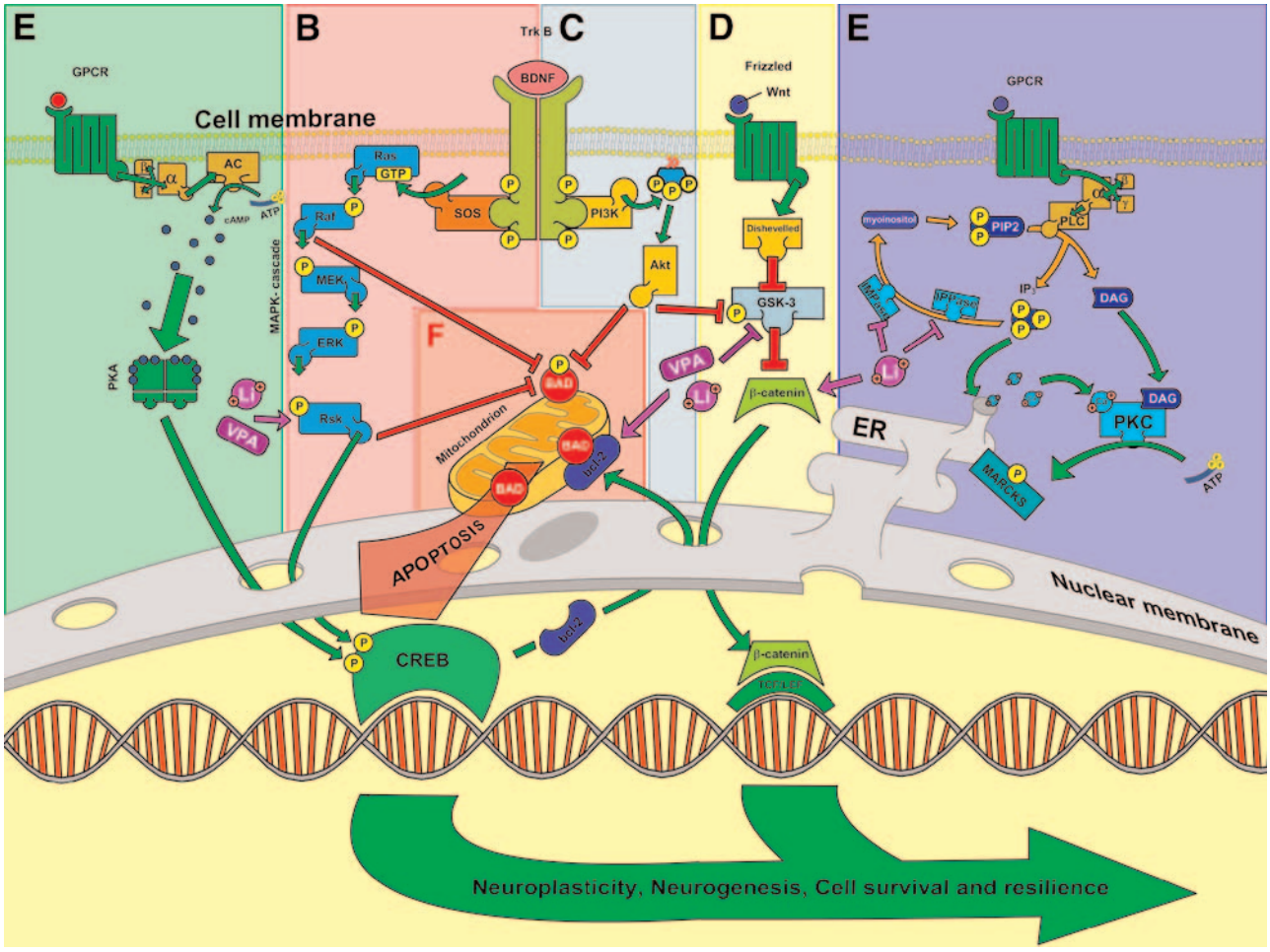
stabilizing signaling activities within an optimal range and preventing fluctuations either above or below this optimum.

Because cAMP can modulate transcription factors that regulate the expression of numerous genes, stabilization of these cAMP fluctuations by lithium might contribute to stabilization of the regulated genes in effected cells. The phosphoinositol signaling cascade (Fig. 1E) mediated by G protein activation of PLC-β is one of the most studied pathways in bipolar research, and evidence exists that at therapeutically relevant concentrations, lithium exerts major effects. As mentioned earlier, lithium directly inhibits IMPase, an enzyme involved in this cascade. PLC catalyzes the hydrolysis of phosphoinositide phosphate (PIP-2) to diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP-3). DAG then activates protein kinase C (PKC; *see* Fig. 1E), which, among many other effects, activates myristoylated alanine-rich C kinase substrate (MARCKS). MARCKS is an acidic protein as well as the most prominent substrate for PKC in the brain. IP-3 binds to the IP-3 receptor, which functions as a calcium channel in the cell; binding IP-3 to its receptor releases intracellular calcium reservoirs from the endoplasmic reticulum. IP-3 binding sets forth downstream effects such as activation of calmodulins and calmodulin-dependent protein kinases and is then recycled back to PIP-2 by the enzymes IMPase (the rate limiting enzyme) and IPPase. Therefore, lithium's direct inhibition of these enzymes (as discussed earlier) suggests that through this mechanism lithium may decrease the availability of myo-inositol and, therefore, the amount of PIP-2 available for G protein-mediated signaling events that depend on this pathway. This led to the inositol depletion hypothesis of lithium's action *(43–48)*. Through these mechanisms, it is suggested that IP-3 and DAG are depleted, thus preventing DAG from exerting its activation on PKC as well as the effects of IP-3 on calcium mobility.

The inositol depletion hypothesis is supported by a great deal of preclinical evidence. Allison and colleagues *(44,45)* first reported the effects of lithium on aspects of phosphoinositol signalling, observing an accumulation of inositol-1-phosphate and a reduction of inositol in rat brain following an acute (nontherapeutic) dose of lithium. Recent reports have also suggested the involvement of the mood stabilizers carbamazepine and valproate in the phosphoinositol cycle; it was demonstrated that carbamazepine and valproate, as well as lithium, increased the spread of growth cones and inhibited their collapse in cells from the rat dorsal root ganglion. Notably, this outcome was prevented by addition of exogenous myoinositol *(15)*. Downstream functions of the phosphoinositol signaling pathway—specifically PKC and PKC signaling (Fig. 1E)—appear to be a target of lithium and valproate as well (ref. *49*; Fig. 3). For example, chronic lithium and valproate treatment decreases the level of PKC isozymes-α and -ε *(50–53)* in cells and treated rodents (*see* below).

The precise mechanisms by which lithium exerts these isozyme-selective actions is unknown, but lithium's effects appear to depend on inhibition of IMPase/IPPase *(49,50)*. Further support for the effect of lithium on PKC is that lithium decreases the levels and phosphorylation of the major PKC substrate MARCKS following chronic lithium and valproate treatment in rats *(14,54–56)*. In cultured cells, this affect appeared to depend on low media inositol concentrations, again implicating lithium's inhibition of IMPase and/or IPPase as a causative factor *(49,55)*.

The fact that lithium and valproate have similar effects is important. A major problem inherent in neuropharmacological research is the difficulty in precisely ascribing therapeutic relevance to any observed biochemical finding, especially in the absence of suitable animal models (and unclear direct targets) for bipolar disorder. As suggested earlier, one approach that has been used is the identification of common biochemical targets, which are modified by drugs that belong to the same therapeutic class (e.g., antimanic agents) but that possess distinct chemical structures (e.g., lithium and valproate) when administered in a "therapeutically relevant" paradigm. Although they likely



do not work by precisely the same mechanisms, identifying the biochemical targets regulated *(49)* in concert by these two chemically distinct agents may provide important clues about molecular mechanisms underlying mood stabilization in the brain. It has been found that the structurally highly dissimilar agent valproate produces strikingly similar effects to lithium on the PKC signaling pathway *(14)*. Notably, lithium and valproate appear to regulate PKC isozymes by distinct mechanisms, with valproate's effects appearing to be largely independent of myo-inositol. This biochemical observation is consistent with the clinical observations that some patients show preferential response to one agent or the other and that one often observes additive therapeutic effects in patients when the two agents are co-administered.

Notably, psychostimulants, which are capable of both triggering manic episodes in susceptible individuals *(57,58)* and inducing manic-like behaviors in rodents *(59,60)*, are known to activate PKC *(61–64)*. Therefore, several independent laboratories have now demonstrated that both acute and chronic amphetamines produce an alteration in PKC activity and its relative cytosol to membrane distribution as well as the phosphorylation of the major PKC substrate GAP-43, which has been implicated in long-term alterations of neurotransmitter release *(61)*. Furthermore, the activation of PKC by psycho-

Fig. 1. Signaling cascades and their involvement in the action of lithium and valproate (valproate acid, VPA). **(A)** Adenylate cyclase (AC) signaling pathway. This pathway is mediated through a G protein-coupled receptor, which activates (via AC) another second messenger called cyclic adeninine monophosphate (cAMP). One of the effects of cAMP is activation of protein kinase A (PKA), an enzyme that phosphorylates many substrates, including cAMP response element-binding protein (CREB). After activation, this protein binds to CRE, a gene sequence found in the promoter of certain genes. **(B)** Extracellular signal-regulated kinase–mitogen-activated protein kinase (ERK–MAPK) pathway. The influence of neurotrophic factors on cell survival is mediated by activation of the MAPK cascade through activation of the neurotrophic factor receptor, also referred to as receptor tyrosine kinases (RTKs). RTKs belong to the tyrosine receptor kinase (Trk) family of receptors. Recruitment of the guanine nucleotide exchange factor Sos results in activation of the small guanosine triphosphate binding protein Ras, which leads to the activation of a cascade of serine/threonine kinases. This includes Raf, MAPK kinase and MAPK (also referred to as ERK). One target of the MAPK cascade is ribosomal S6 kinase (RSK), which influences cell survival by phosphorylating and inactivating the pro-apoptotic factor Bcl-xl/Bcl-2 associated death promoter (BAD). The ribosomal S6 kinases also phosphorylates CREB, thereby increasing the expression of the anti-apoptotic factor Bcl-2 and brain-derived neurotrophic factor. These mechanisms underlie many of the long-term effects of neurotrophins, including neurite outgrowth, cytoskeletal remodelling, and cell survival. Lithium and valproate appear to activate the ERK–MAPK pathway. **(C)** Phosphatidylinositol-3-kinase (PI-3-K) pathway. This pathway can be also be activated by RTKs. One way that PI-3-K signals cells to survive and grow is by indirectly activating protein kinase Akt. Akt phosphorylates a variety of target proteins. Some of them are the same that are phosphorylated via the ERK–MAP pathway (e.g., BAD). A main substrate of Akt is Glycogen synthase kinase-3 (GSK-3). **(D)** GSK-3 and Wnt pathway. GSK-3 mediates numerous signaling pathways, including the Wnt pathway, neurotrophic pathways, and the insulin/PI-3-K pathway. In the Wnt signaling pathway, Wnt glycoproteins interact with the frizzled family of receptors to stimulate the disheveled-mediated inactivation of GSK-3 and activation of the transcription factor β-catenin. Following GSK-3 inhibition, nondegraded (nonphosphorylated) β-catenin binds to lef/tcf transcription factors, targeting transcription of specific genes. Disheveled inactivation of GSK-3 results via interactions with a protein complex that contains adenomatous polyposis coli, axin, and FRAT1. Lithium is a direct inhibitor of GSK-3; both lithium and valproate increase β-catenin levels. **(E)** Phosphoinositol signaling pathway and protein kinase C (PKC). After ligand binding to a G protein-coupled receptor, the three subunits of the G protein dissociate from both each other and the receptor, and this activation induces phospholipase C (PLC) hydrolysis of phosphoinositide phosphate (PIP)-2 to diacylglycerol (DAG) and inositol-1,4,5-triphosphate (IP-3). DAG and IP-3 function as second messenger. DAG activates PKC, an enzyme that has many effects, including activation of myristoylated alanine-rich C kinase substrate (MARCKS). IP-3 binds to the IP-3 receptor, which also functions as a calcium channel in the cell. IP-3 is recycled back to PIP-2 by the enzymes inositol-monophosphatase and inositol-polyphosphate 1-phosphatase, both of which are direct targets of lithium. **(F)** Mitochondria, calcium, and Bcl-2. Bcl-2 attenuates apoptosis by sequestering proforms of caspases (death-driving cysteine proteases); by preventing the release of mitochondrial apoptogenic (programed cell death) factors such as calcium, cytochrome c, and apoptosis-inducing factor into the cytoplasm; and by enhancing mitochondrial calcium uptake. Before classic signs of apoptosis occur, mitochondria undergo major changes in membrane integrity, such as disruption of the inner transmembrane potential and the release of intermembrane proteins through the outer membrane. Bcl-2 stabilizes mitochondrial membrane integrity and prevents opening of the permeability transition pore, a key event in cell death. Lithium and valproate increase Bcl-2 levels.

stimulants has been shown to play an important role in facilitating the release of dopamine, one of the neurotransmitters implicated most commomly in the pathogenesis of mania *(58)*. Therefore, together, the biochemical data suggest that two structurally dissimilar antimanic agents, lithium and valproate, attenuate PKC function in a therapeutically relevant time frame, whereas promanic psychostimulants activate PKC. These data suggest that PKC modulation plays a critical role in the treatment of mania.

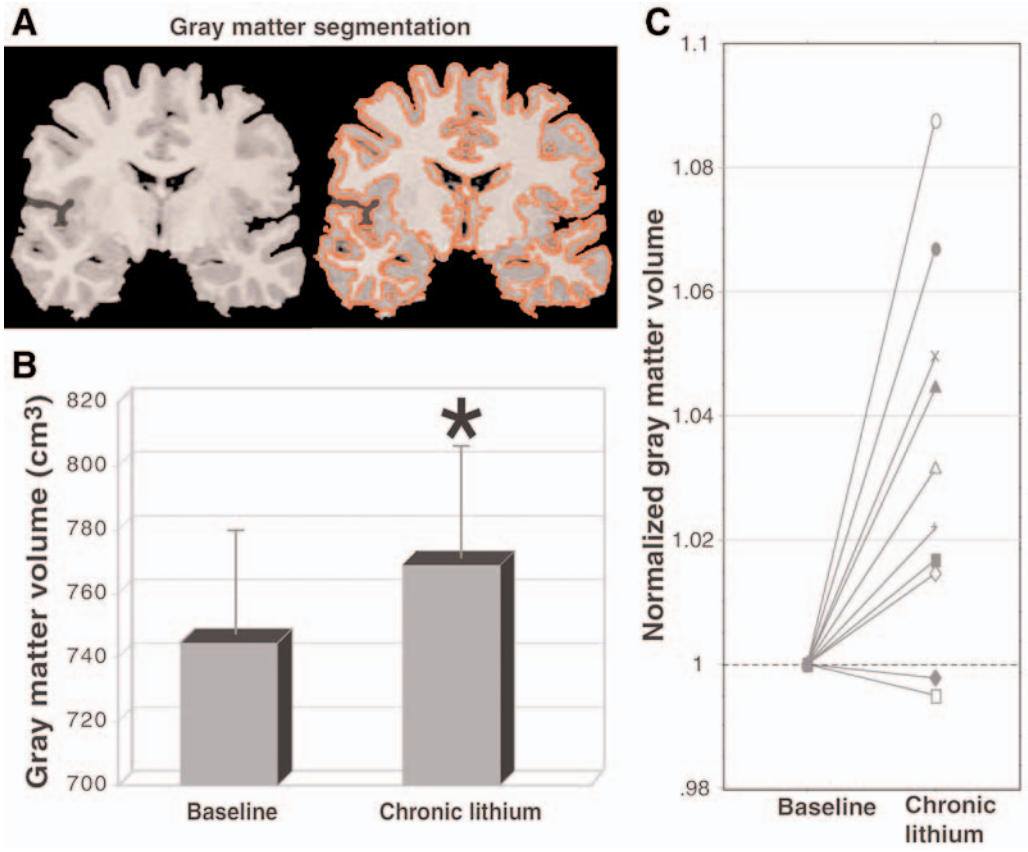


Fig. 2. Lithium-induced increase in human brain gray matter. Brain gray matter is increased following 4 wk of lithium administration at therapeutic levels in patients with bipolar disorder. **(A)** A slice of three-dimensional tric magnetic resonance imaging (MRI) data that was segmented by tissue type using quantitative methodology to determine tissues at each scan time point. Brain tissues were examined using high-resolution three-dimensional MRI (124 1.5-mm-thick coronal T_1-weighted spoiled gradient echo images) and validated quantitative brain tissue segmentation methodology by which to identify and quantify the various components, including total brain white and gray matter content. Measurements were made at baseline (medication-free, after a minimum 14-d washout) and then repeated after 4 wk of lithium at therapeutic doses. **(B, C)** Chronic lithium significantly increases total gray matter content in the human brain of patients with bipolar disorder. No significant changes were observed in brain white matter or quantitative measures of regional cerebral water. (Modified and reproduced with permission from ref. *187*.)

Although the biochemical studies on the PKC signaling cascade are compelling, ascribing a role of this pathway to the pathophysiology/treatment of bipolar disorder clearly requires elaboration at the behavioral level in animal models of affective disorders. In this context, Birnbaum and associates *(65)* recently demonstrated that either direct or indirect activation of PKC dramatically impaired the cognitive functions of the rhesus monkey prefrontal cortex. Thus, exposure stress activated PKC and resulted in prefrontal dysfunction, whereas inhibiting PKC (including indirectly with mood stabilizers) protected cognitive function.

A clinical study these supports these extensive preclinical. Specifically, lithium treatment results in myo-inositol reductions in the frontal cortex of bipolar patients after 5 d of treatment

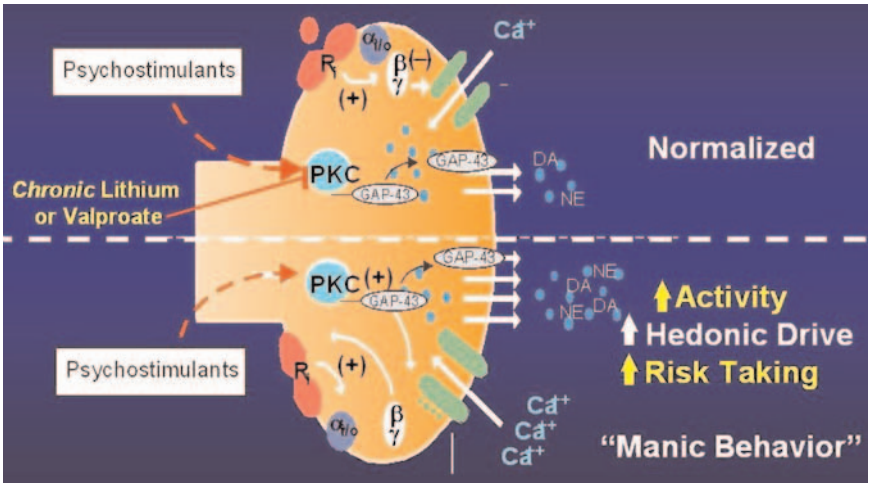


Fig. 3. Schematic representation of the potential involvement of protein kinase C (PKC) in the pathophysiology and treatment of acute mania. **Bottom,** Psychostimulants, which induce mania, are now known to activate PKC. PKC activation facilitates the release of catecholamines via diverse mechanisms, including regulating autoreceptor-coupled G proteins, calcium channels, and phosphorylating GAP-43. Phosphorylation of GAP-43 leads to its membrane translocation, where it plays a critical role in neurotransmitter release. The amphetamine-induced PKC activation, GAP-43 translocation, and catecholamine release leads to increased activity, increased hedonic drive, and increased risk taking in animal models—all facets of the manic behavior. **Top,** Chronic lithium and valproate attenuate PKC activity, which may play an important role in their antimanic effects. A direct acting PKC inhibitor may thus not only attenuate mania but may do so rapidly.

(66). However, at 5 d, the clinical state of the patients was completely unchanged, indicating that reduction of myo-inositol levels *per se* is not associated with therapeutic response but may initiate a cascade of secondary changes in the phosphoinositol signaling pathway and gene expression in the CNS *(66)*. These downstream events may ultimately be responsible for the therapeutical effects of lithium. To date, there has been only a limited number of studies directly examining PKC in bipolar disorder (ref. *67*; Table 1). Although undoubtedly an oversimplification, particulate (membrane) PKC is sometimes viewed as the more active form of PKC; thus, an examination of the subcellular partitioning of this enzyme can be used as an index of the degree of activation.

Friedman et al. *(68)* investigated PKC activity and PKC translocation in response to serotonin in platelets obtained from subjects with bipolar disorder before and during lithium treatment. They reported that the ratios of platelet membrane-bound to cytosolic PKC activities were elevated in the manic subjects. Additionally, serotonin-elicited platelet PKC translocation was enhanced in those subjects. In postmortem brain tissue from patients with bipolar disorder, Wang and Friedman *(69)* measured PKC isozyme levels, activity, and translocation; they reported increased PKC activity and translocation in bipolar disorder brains compared to controls, effects that were accompanied by elevated levels of selected PKC isozymes in the cortex of subjects with bipolar disorder.

The earlier discussion detailed the evidence that mood stabilizers may target the phosphoinositol/PKC and AC signaling pathway. Because of the pivotal role of the PKC signaling pathway in the regulation of neuronal excitability, neurotransmitter release, and long-term synaptic events, it was postulated that the attenuation of PKC activity may play a role in the antimanic effects of lithium and valproate

Table 1
Evidence Implicating PKC in the Pathophysiology and Treatment of Bipolar Disorder

- Increased membrane/cytosol PKC partitioning in platelets from manic subjects; normalized with lithium treatment
- Increased PKC activity and translocation in obtained at postmortem from patients with bipolar disorder compared to controls
- Amphetamine produces increases in PKC activity and GAP-43 phosphorylation (implicated in neurotransmitter release)
- PKC inhibitors block the biochemical and behavioral responses to amphetamine and cocaine and also block cocaine-induced sensitization
- Lithium and valproate regulate PKC activity, PKC-α, PKC-ε, and MARCKS
- Preliminary data suggest that PKC inhibitors may have efficacy in the treatment of acute mania

PKC, protein kinase C; MARCKS, myristoylated alanine-rich C kinase substrate.

(*see* Fig. 3). In support of this contention, a pilot study found that tamoxifen (a nonsteroidal anti-estrogen known to be a PKC inhibitor at higher concentrations) may possess antimanic efficacy (discussed in more detail later) *(198)*.

These pathways have long been a focus of research on mood stabilizers. Newer targets for the mechanisms of action of lithium and valproate include glycogen synthasekinase-3 (Wnt signaling pathway) and various neurotrophic signaling pathways that are explained in more detail in the following sections.

Glycogen Synthase Kinase-3 and the Wnt Pathway

Glycogen Synthase Kinase-3: A Direct Target of Lithium

In 1996 it was known that administration of lithium to developing *Xenopus* embryos had the same effect (i.e., duplication of the dorsal axis) as downregulation of GSK-3 *(70,71)*. This parallel in a developmental model led to studies directly examining the effects of lithium on GSK-3, demonstrating that GSK-3 was directly inhibited by lithium (ref. *72*; Fig. 1D). Lithium was initially found to inhibit GSK-3 with a Ki of 1 to 2 m*M* (serum therapeutic range: 0.6–1.2 m*M*; (refs. *72* and *73*). However, evidence showing that lithium competes with magnesium *(21,74)* suggests that the original studies using higher than physiological levels of magnesium may have underestimated the degree of inhibition.

Indeed, recent studies have suggested a significant inhibition of this enzyme in the rodent brain at therapeutic serum lithium levels during long-term treatment. For example, Gould et al. *(36)* found that 9 d of lithium treatment increased cytosolic protein levels of β-catenin (a transcription factor regulated directly by GSK-3). This protein level increase was accompanied by a small but significant decrease in β-catenin messenger RNA (mRNA) levels, further suggesting that lithium exerted its actions post-translationally by inhibiting GSK-3 (the mRNA changes reflecting cellular compensation) *(36)*. Confirmatory findings reporting that chronic lithium activates β-catenin-dependent transcription in the mouse brain have been published recently *(75)*. Furthermore, it was also reported that 3 wk of lithium treatment decreased brain levels of amyloid-β peptide in AP-Swedish/Tg2576 mice (a model of familial Alzheimer's disease). Given lithium's effect on amyloid-β accumulation in cell culture *(76)*, this finding likely results from inhibition of GSK-3. Furthermore, GSK-3 can be indirectly inhibited through phosphorylation mechanisms. Through a "feedback" mechanism, in vivo inhibition of GSK-3 results in an increase in serine phosphorylation. This finding, originally observed by Chuang and colleagues *(77)*, has been replicated by several groups. Proposed mechanisms include GSK-3-regulated decreased activity of protein phosphatase 1 *(78)*, increased activity of

PKC *(79)*, or increased Akt activity *(77)*. Therefore, moderate direct inhibition by lithium is amplified through an increase in serine 21/9 phosphorylation (the inhibitory serine sites on GSK-3α and -3β, respectively).

GSK-3 is a serine/threonine kinase that is both normally highly active in cells and deactivated by signals originating from numerous signaling pathways (e.g., the Wnt pathway, phosphatidylinositol-3-kinase (PI-3-K) pathway, PKA, PKC, and so on; *see* Fig. 1D). It is found in two forms, α and β, which have similar (but not always identical) biological functions. Cellular targets of GSK-3 are numerous and often depend on the signaling pathway that is acting on it (because of cellular localization and regional sequestration). For example, Wnt pathway inhibition of GSK-3 activates the transcription factor β-catenin, whereas in the insulin–PI-3-K signaling pathway, inhibition of GSK-3 results in activation of the enzyme glycogen synthase. Targets of GSK-3 include transcription factors (β-catenin, CREB, c-Jun), proteins bound to microtubules (Tau, MAP1B, kinesin light chain), cell-cycle mediators (cyclin D, human ninein), and regulators of metabolism (glycogen synthase, pyruvate dehydrogenase), among others (for review, *see* refs. *80* and *81*).

As a component of many signaling pathways, with multiple cellular targets to choose from, GSK-3 can regulate a diverse array of cellular processes, such as glycogen synthesis, gene transcription, events related to synaptic plasticity, apoptosis, and the circadian cycle (for review, *see* refs. *82–85*). Although many of these functions are probably critically important to both cellular and organism functioning, GSK-3 is currently receiving foremost interest as a regulator of apoptosis and cellular resilience. Generally, increased activity of GSK-3 is pro-apoptotic, whereas inhibition of GSK-3 attenuates or prevents apoptosis (for review, *see* refs. *83* and *85*). As discussed later, evidence suggests an association between mood disorders and impairments of neuroplasticity and cellular resilience *(86)*. In this regard, lithium has effects suggestive of neuroprotection both clinically and in rodent- and cell-based models (for review, *see* ref. *86*). Lithium may at least partly exert these neuroprotective effects by inhibition of GSK-3 *(83,85)*.

GSK-3 also appears to represent a *therapeutic* target of lithium. Recent evidence has suggested that the behavioral effects of lithium, at least in rodent models, may also result from inhibition of GSK-3. Three groups have found that administration of GSK-3 inhibitors results in antidepressant-like effects in the forced-swim-test paradigm following either intracerebral ventricle injections in mice *(87)*, peripheral administration to rats *(88)*, or lithium administration to mice *(75)*. Furthermore, O'Brien and colleagues *(75)* recently examined the behavioral effects of knocking-out a single copy of GSK-3β gene and observed in these animals the same antidepressant-like behavior induced by both alternate pharmacological inhibition and lithium administration (i.e., increased mobility in the forced-swim test). To further support the hypothesis that the effects of antidepressants may be mediated in a GSK-3-dependent manner, Li and colleagues *(89)* reported that inhibitory (serine 9) phosphorylation of GSK-3 acutely increased by increasing serotonin levels through various pharmacological mechanisms (including common antidepressants). Therefore, GSK-3 inhibition may represent a therapeutically relevant downstream consequence of antidepressant drugs that initially target serotonin levels.

Psychostimulant-induced hyperactivity is an established rodent model of mania. This behavior is reproducibly attenuated by numerous mood stabilizers, including lithium, anticonvulsants, and antipsychotics. Beaulieu et al. *(90)* recently reported that dopamine-dependent activity increases in mice are mediated in large part by a GSK-3-dependent mechanism. They reported that both lithium and alternate GSK-3 inhibitors attenuated the hyperactivity in mice that lacked the dopamine transporter. They also found that amphetamine administration to wild-type mice resulted in a decrease in the inhibitory phosphorylation of GSK-3 and that mice heterozygous for GSK-3 had an attenuated response to amphetamine adminis-

tration. Accordingly, peripheral administration of a GSK-3 inhibitor decreased amphetamine-induced hyperactivity in rats *(88)*. Together, these data support the possibility that inhibition of GSK-3 may represent lithium's antimanic as well as its antidepressant target. It is critical to future understanding of mood disorder etiology to determine which GSK-3 target(s) is responsible for behavior in models of both mania and depression.

The Wnt Signaling Pathway: A Target of Valproate As Well

Lithium's activation of the Wnt signaling pathway (Fig. 1D) is well-established as through inhibition of GSK-3 *(72,83,91)*. However, there is also evidence that valproate is an activator of the Wnt signaling pathway *(83,92,93)*. For example, in human neuroblastoma SH-SY5Y cells, incubation with 0.6 m*M* of valproate resulted in a decrease in phosphorylation of GSK-3 substrates *(93)*. Similarly, valproate treatment prevented the phosphorylation of a GSK-3 target MAP1B in developing neurons in culture *(94)*. Furthermore, 1-d (and longer) treatment of SH-SY5Y cells with a therapeutically relevant concentration of valproate resulted in a significant increase in both nuclear and cytoplasmic β-catenin protein levels *(93)*. Valproate also increased lef/tcf-mediated reporter gene expression in neuro 2A cells *(92)*. Valproate exerted effects on other pathways regulated by GSK-3; specifically, it prevented GSK-3-mediated cell death *(95)* and counteracted the effects of GSK-3 on CREB DNA binding activity *(96)*. Similarly to the effects of lithium, treatment of rats with therapeutic concentrations of valproate for 9 d increased cytoplasmic β-catenin levels in the cortex *(36)*.

Neurotrophic Signaling Pathways

The following section describes the pharmacological evidence and rationale for the involvement of neurotrophic pathways in the treatment of bipolar disorder. To understand the importance of these neuroprotective and neurotrophic actions as mechanisms of mood-stabilizing action in the brain, we first focus on clinical evidence in bipolar disorder for actual impairments in cellular resilience (ability of the cell to handle both physiological and pathophysiological loads/demands) and in neuroplasticity (diverse processes by which the brain perceives, adapts to, and responds to various internal and external stimuli, including alterations of dendritic function, synaptic remodeling, neurite extension, synaptogenesis, and neurogenesis).

Clinical Evidence for Cell Death, Cell Atrophy, and Impairments of Cellular Resilience in Bipolar Disorder

Methodologies to investigate structural and functional deficits within the human brain have exponentially increased in recent years, allowing for a more complete and intensive analysis of potential deficiencies in the brains of patients suffering from neuropsychiatric disorders. Therefore, it is now possible to discern relatively small differences in cerebral blood flow, receptor density, or macroscopic brain structure. Similarly, postmortem studies are relying on methodologies, which, although they are not perfect, allow for unbiased observations of cell density, morphology, and size. Recent years have also seen the size and availability of postmortem brains repositories for patients with mood disorders increase. The functional and morphometric findings in bipolar disorder have been extensively reviewed elsewhere *(97–106)*; here, we provide a brief overview of these findings.

Positron emission tomography imaging studies have revealed multiple alterations of regional cerebral blood flow and glucose metabolism in limbic and prefrontal cortical (PFC) structures in patients with mood disorders. Overall, these findings implicate limbic–thalamic–cortical and limbic–cortical–striatal–pallidal–thalamic circuits, involving the amygdala, orbital, and medial PFC as well as anatomically related parts of the striatum and thalamus in the pathophysiology of mood disorders (for review,

see refs. *97–100*). Recent morphometric magnetic resonance imaging (MRI) and postmortem investigations have also demonstrated abnormalities of brain structure that are likely to contribute to the corresponding abnormalities of metabolic activity (discussed in refs. *99–101*). Structural imaging studies demonstrate reduced gray matter volumes in areas of the orbital and medial PFC and temporal lobe as well as enlargement of third ventricle in subjects with mood disorders compared to healthy controls (reviewed in refs. *102–104*). Also consistent is the increased presence of white matter hyperintensities in the brains of elderly patients with depression and in both young and elderly patients with bipolar disorder (reviewed in refs. *84* and *107*). Although the cause of white matter hyperintensities in mood disorders is unknown, their presence—particularly in the brains of young patients with bipolar disorder—suggests importance in the pathophysiology of the disorder *(84,108)*.

Relatively recent postmortem neuropathological studies are complementary, showing reductions in cortex volume as well as region- and layer-specific reductions in number, density, and/or size of neurons and glial cells in the subgenual PFC, orbital cortex, dorsal anterolateral PFC, amygdala, and basal ganglia and dorsal raphe nuclei in individuals with bipolar disorder and other severe mood disorders compared with controls (reviewed in refs. *101,105,* and *106*).

Note, however, that it is not currently known if these alterations—whether functional and structural imaging or postmortem—constitute developmental abnormalities conferring vulnerability to severe mood episodes, compensatory changes to other pathogenic processes, the sequelae of recurrent affective episodes, or solely epiphenomenon lacking real significance in the pathophysiology or pathogenesis of these disorders. Understanding these issues partly depends on experiments that both delineate the onset of such abnormalities within the illness course and determine whether they antedate mood episodes in high-risk individuals. Furthermore, there is not total reproducibility among either the neuro-imaging or postmortem studies. This may be representative of variations of experimental design (including medication effects), an effect of low sample size, or difficulty in clearly defining disease phenotypes within a heterogeneous group of disorders. Therefore, further study is required to determine if more rigorously defined subtypes of bipolar disorder and other mood disorders are associated with any particular or specific alteration *(84)*.

The anatomical abnormalities described earlier indicate cellular and circuit functional impairment as an important feature of bipolar disorder. These changes most likely arise from a complex pathophysiology involving multiple susceptibility (and likely protective) genes as well as environmental factors. Evidence suggests that stressful life events may trigger the onset of affective disorders in genetically susceptible individuals *(109)*. In rodents, certain stressors produce dendritic atrophy, death, or endangerment (priming the substrate to be vulnerable to other pathophysiological insults) of hippocampal CA3 pyramidal neurons *(110–113)*. Furthermore, stressors decrease levels of neurotrophins (NTs; ref. *110*) and reduce the number of newborn hippocampal granule cells *(114–116)*, a process called neurogenesis (discussed in more detail later).

The brain continually responds to internal and external stimuli by changing the properties of neural circuits, a phenomenon called neuroplasticity. All complex psychological and cognitive processes, as well as diverse neurovegetative functions, rely on these mechanisms. Cellular correlates for neuroplasticity are axon and dendrite growth, synaptogenesis and synaptic remodeling, long-term potentiation (LTP), and the generation of new neurons. Although neuroprotection protects the brain against cell loss and atrophy caused by the pathophysiology of the disorder, a repair of already existing damage depends on mechanisms of neuroplasticity, increasing trophic support, and cell proliferation. On the molecular level, these events are controlled by complex intracellular signaling cascades that process incoming information into adaptations of protein activity and genetic response. Recent

Table 2
Neurotrophic and Neuroprotective Effects of Bcl-2 and Lithium

Bcl-2	Lithium
Protect against the lethal effects of a variety of reactive oxygen species	**Cultured cells (human and rodent) in vivo**
Protects against the MPTP and AMPA neurotoxicity	Protects against glutamate, NMDA toxicity
Protects against growth factor deprivation	Protects against calcium toxicity
Protects against the effects of ionizing radiation	Protects against MPP 1 toxicity
Protects against glucocorticoid toxicity	Protects against β-amyloid toxicity
Reduces neuropathology after focal ischemia	Protects against aging "toxicity"
Reduces neuropathology after traumatic brain injury	Protects against growth factor deprivation
Prevents axotomy-induced motor neuron death	**Rodent brain in vivo**
Attenuates motor neuron degeneration in a transgenic animal model of ALS	Protects against cholinergic system lesion toxicity
Regulates neurite sprouting and outgrowth and increases axonal growth rate	Protects against radiation injury
Promotes regeneration of axons in the mammalian central nervous system	Protects against MCA occlusion toxicity
	Protects against quinolinic acid toxicity
	Enhances hippocampal neurogenesisin the adult rodent brain
	Human effects
	Patients with bipolar disorder chronically treated with lithium or valproate do not show reduced subgenual prefrontal cortex
	Increases NAA levels in the brains of patients with bipolar disorder
	Increases gray matter s in the brain of patients with bipolar disorder

MPTP, 1,methyl-4-phenyl-1,2,3,6-tetrahydropyridine; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-D-aspartate; MPP, 1-methyl-4-phenylpyridine; ALS, amytrophic lateral sclerosis; MCA, major coronary artery; NAA, *N*-acetyl-aspartate.

evidence has demonstrated that these processes are targeted by lithium and valproate, and the observation that their effects occur only after chronic (days to weeks) administration strongly supports the hypothesis that these may be relevant underlying therapeutic mechanisms.

The following section summarizes the evidence of the neuroprotective and neurotrophic actions of lithium and valproate.

Neuroprotection

Several preclinical studies have shown that lithium and valproate have neuroprotective properties. Lithium has been documented to protect cells in culture against deleterious effects of various neurotoxic conditions such as serum and nerve growth factor (NGF) deprivation *(117)*, oxygen and glucose deprivation *(118)*, low K^+ *(119)*, low Na^+ *(120)*, glutamate- and *N*-methyl-D-aspartate (NMDA)-induced excitotoxicity *(77, 121-124)*, ceramide *(125)*, anticonvulsants *(126)*, aging *(127)*, and β-amyloid *(86,128,129)* (Table 2). Furthermore, as discussed in more detail later, in vivo studies in various animal models have shown neuroprotective properties of lithium and valproate. Furthermore, chronic lithium and valproate administered at therapeutic concentrations increased the expression and protein levels of a major neuroprotective and anti-apoptotic protein (Bcl-2) in the rat frontal cortex and hippocampus. Lithium also robustly increases Bcl-2 expression in the striatum (*86,130*; Table 2).

In a rodent model for stroke using middle cerebral artery occlusion, lithium and valproate markedly reduced neurological deficits and decreased brain infarct size when adminsitered before *(131)* or following middle cerebral artery occlusion *(132,133)*. Additionally, in a rat model of Huntington's disease, lithium significantly reduced brain lesions resulting from infusion of quinolinic acid, an excitotoxin *(134)*. In a mouse model for Alzheimer's disease, lithium blocked the accumulation of amyloid β-peptides in the brains of mice that overproduce APP (ref. *76*; Table 2).

Neurogenesis

It is now known that neurogenesis (the birth of new neurons) not only occurs in the brain during development but also continues into adulthood in mammalian species, including humans *(135–139)*. Stress induces the downregulation of neurogenesis and chronic, but not acute, treatment of adult rats with various antidepressants increases neurogenesis as detected by 5-bromo-2'-deoxyuridine (BrdU) labeling of proliferating neurons *(140–142)*. BrdU—a thymidine analog—is incorporated into the DNA during DNA synthesis and can be immunohistochemically detected, thus serving as marker for newborn cells. It was recently shown that disruption of neurogenesis in different mouse models blocks the behavioral response to antidepressants *(143)*. Lithium stimulates progenitor cell proliferation in cultured brain neurons *(144)* and has been shown to increase BrdU-positive cells in the adult rat hippocampus by approx 25% *(145)*. Recent data also suggest that valproate also has effects on neurogenesis, specifically increasing the proliferation of rat cerebral cortical cells in culture and the number of BrdU-positive neurons in the mouse dentate gyrus *(146)*.

Effects of Mood Stabilizers on Neurotrophic Signaling Pathways

Neurotrophic factors were first characterized for regulating neural growth and differentiation during development *(147)* but are now known to be potent regulators of plasticity, survival, and differentiation of adult neurons and glia *(148,149)*. Brain-derived neurotrophic factor (BDNF), one of the most prevalent neurotrophic factors in adult brain, was shown to be downregulated by chronic and acute stresses in rodent hippocampus *(150–153)*. Conversely, chronic—but not acute—administration of virtually all classes of antidepressants increases BDNF expression in these regions and can prevent the stress-induced decreases of BDNF levels *(154)*. Evidence also exists to indicate that antidepressants may increase BDNF levels in the brains of humans *(155)*. Furthermore, administration of BDNF or a related NT (NT-3) into the hippocampus of rats results in an antidepressant effect in behavioral paradigms for depression *(156)*.

Recent studies have shown that chronic treatment with either lithium or valproate increases the levels of immunoreactivity of BDNF in the rat frontal cortex *(59,157,158)*. Thus, these mood stabilizers may enhance LTP and other forms of synaptic plasticity that have been connected with BDNF. Because sufficient time would be required for levels of BDNF to increase and to exert their neurotrophic effects, these findings may also explain the delay of onset of mood-stabilizer therapeutic response. Indeed, it was recently shown that chronic lithium treatment enhanced LTP in the dentate gyrus of young and aged rats *(159,160)*.

Extracellular Signal-Regulated Kinase–Mitogen-Activated Protein Kinase Pathway

All known neurotrophins (such as NGF, BDNF, NT-3, NT-4/5, and NT-6) act via a specific receptor tyrosine kinase (RTK) *(161)* belonging to the tyrosine receptor kinase (Trk) family of receptors (TrkA, TrkB, TrkC, p^{75}). Ligand binding to RTKs (Fig. 1B) causes the activation of intracellular cascades regulating downstream targets (including enzymes) as well as gene regulatory proteins in the nucleus. The resulting changes in gene expression and protein activity

lead to complex changes in cell behavior, such as proliferation, differentiation, and cell survival, as well as the modulation of synaptic transmission and neuroplasticity *(162,163)*.

Two of the main pathways activated by RTKs have been demonstrated to be influenced by lithium and valproate: the extracellular signal-regulated kinase–mitogen-activated protein kinase cascade (ERK–MAPK pathway; Fig. 1B) and the PI-3-K (Fig. 1C). Ligand binding to RTKs activates the small G protein Ras by exchanging guanine diphosphate to guanine triphosphate.

Ras (Fig. 1B) activates the ERK–MAPK pathway, which consists of the kinases Raf, MEK, and ERK. Among the targets of Erk are the ribosomal S6 kinases (RSKs), which have been shown to phosphorylate several transcription factor and proteins. One of the transcription factors activated by RSKs is CREB. CREB activation increases the expression of the anti-apoptotic protein Bcl-2 as well as the NT BDNF. A different mechanism by which RSK inhibits apoptosis is the phosphorylation, and thereby inactivation, of the pro-apoptotic protein Bcl-xl/Bcl-2 associated death promoter (BAD).

At therapeutically relevant concentrations, lithium and valproate activate the ERK–MAPK pathway (measured by the phosphorylation of ERK and RSK) in human neuroblastoma SH-SY5Y cells, primary cortical cells, cerebellar granule cells, and in rodent cortex *(59,86,129, 164)*. In addition to previously described BDNF findings, lithium has been shown to increase Bcl-2 levels in rat cerebellar granule cells *(124)*; SH-SY5Y cells *(165)*; retinal ganglion cells *(166)*; C57BL/6 mouse brains *(145)*; and rat frontal cortex, striatum, and hippocampus *(130,167)*. Although valproate has not been as well-studied, it increases Bcl-2 in the rat frontal cortex and hippocampus following chronic treatment *(130)*.

PI-3-K/Akt

Another major intracellular signaling pathway that can be activated by RTKs involves PI-3-K (Fig. 1C). PI-3-K catalyzes the phosphorylation of PI, a unique membrane lipid that can undergo reversible phosphorylation. Phosphorylated PIs serve as docking sites for intracellular signaling proteins, bringing these proteins together into signaling complexes that relay the signal from the plasma membrane into the cell. One way that PI-3-K signals cells to survive and grow is by indirectly activating the protein kinase Akt. Akt phosphorylates various target proteins, some of which are identical to those phosphorylated via the ERK–MAPK pathway (e.g., BAD), and normally stimulates cells to undergo programmed cell death (apoptosis).

A main substrate of Akt is GSK-3 (Fig. 1). The inhibition of GSK-3 by Akt controls important aspects of cell plasticity and death. As discussed earlier, lithium is a direct inhibitor of GSK-3, and valproate also appears to have inhibitory effects on GSK-3 *(21,168)*. As discussed earlier, lithium and valproate both have been shown to inhibit the activity of GSK-3. Additionally, lithium has been shown to increase the activity of PI-3-K *(77)* and to affect two kinases downstream of PI-3-K—namely, PKC and Akt *(77,79)*. Additional studies are necessary to determine whether the Akt activation observed in studies in vitro *(77,129)* occurs in vivo, thus becoming therapeutically relevant.

Mitochondria, Calcium, and Bcl-2

The principal functions of the mitochondria (Fig. 1F) are to produce ATP (and therefore energy), to regulate apoptosis (programmed cell death), and to buffer intracellular calcium (Table 3). High concentrations of calcium overwhelm the mitochondria in their capacity to buffer calcium, leading to depolarization of the mitochondrial membrane potential. Through loss of the membrane potential, mitochondria are not able to further sustain respiration and production of energy. Depolarization combined with the excessive generation of free radicals (reactive oxygen species [ROS]) induces collapse of mitochondrial membrane potential and leads to apoptosis.

Table 3
Take-Home Message

- Lithium and valproate increase the expression of the cytoprotective protein Bcl-2 in neuronal cell cultures and rat frontal cortex
- Lithium and valproate activate the ERK–MAPK pathway in rat frontal cortex and hippocampus
- Valproate promotes neurite outgrowth
- Lithium inhibits GSK-3β and increases β-catenin levels
- Lithium exerts neurprotective effects against diverse insults
- Lithium increases the levels of NAA in human brain
- Lithium increases gray matter volumes in human brain

ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; GSK-3β, glycogen synthase kinase-3 beta; NAA, *N*-acetyl-aspartate.

Recent findings have suggested that bipolar disorder may be related to mitochondrial dysfunction (for review, *see* ref. *169*). Mitochondrial dysfunction could be caused by accumulation of mitochondrial DNA deletions, which leads to calcium dysregulation by the affected mitochondria and to altered calcium signaling. Calcium levels in platelets or leukocytes of patients with bipolar disorder have been shown to be elevated, a finding that might be explained by a genetically or posttranscriptionally modified buffer capacity of mitochondria in patients with bipolar disorder.

Some evidence exists that mood stabilizers might stabilize mitochondrial function by enhancing the mitochondria in their bio-energetic function and by avoiding apoptosis. The impact of mood stabilizers on mitochondrial function could have a beneficial effect on the symptoms of bipolar disorder and may have a long-term supportive effect on neurotrophic and neuroprotective actions. Cell culture studies have demonstrated that chronic lithium administration blocks a toxin-stimulated increase in calcium concentration and free radicals (ROS) production *(170)*, prevents loss of mitochondrial membrane potential *(170)*, and prevents increase of calcium concentration through overstimulation of the NMDA receptor via glutamate *(121,122,129)*. Chronic valproate blocks formation of ROS in cortical neurons treated with the oxidant Fe3 *(171)*. Preliminary findings from our lab have shown increased mitochondrial membrane potential in cell cultures treated with lithium and increased respiration ratios in isolated mitochondria of neuronal cells treated with lithium or valproate (unpublished data).

Increasing evidence suggests that mitochondria may be integrally involved in the general processes of synaptic plasticity. In a detailed investigation of the relative roles of mitochondrial and ER Ca^{2+} buffering, the dendritic mitochondrion were found to rapidly accumulate Ca^{2+}, whereas the endoplasmic reticulum displayed a more delayed increase in Ca^{2+} during high-frequency stimulation *(172)*. Furthermore, increased synaptic activity was shown to induce the expression of mitochondrial-encoded genes, suggesting that the regulation of metabolism is an important component in the long-term regulation of synaptic strength *(173)*. This regulation occurred even with stimulations that were under the threshold for LTP induction, suggesting that a sort of "metabolic priming" may occur. Together, these findings suggest that mitochondrial Ca^{2+} sequestration has a key role in modulating the tone of synaptic plasticity in various anatomical regions. This buffering appears to "backload" synaptic transmission by preventing excessive or asynchronous release during, and preserving or increasing the synaptic response following, high-frequency stimulation. Additionally, energy metabolism and its regulation are likely to be highly involved in long-lasting changes in synaptic strength. (For further discussion of recent research into the mitochondrion's role in synaptic plasticity, *see* ref. *174*.)

The earlier mentioned neurotrophic effects of lithium and valproate on mitochondrial function may be mediated by Bcl-2 (Fig. 1F). Bcl-2 is a major neuroprotective protein and is one of the members of the Bcl-2 family, which consists of anti-apoptotic (Bcl-2 and Bcl-x) and pro-apoptotic (Bad/Bik, Bax, Bak, tBid, Bim) proteins. These proteins are localized to the membranes of the mitochondrion, endoplas-

mic reticulum, nucleus, and golgi. Bcl-2-specific action is to block the mitochondrial program for apoptosis.

As discussed earlier, many findings show that upregulation of Bcl-2 occurs in vivo and in vitro following chronic treatment with lithium (Table 2) or valproate *(124,130)*. This is expected to stabilize mitochondrial function and, therefore, protection from altered energy levels and apoptosis. Bcl-2 increases the maximal capacity of mitochondria to accumulate Ca and prevents collapse of the mitochondrial membrane potential *(175)*. Bcl-2 also inhibits the release of cytochrome c into the intermembrane space of the mitochondria, thus preventing apoptosis (ref. *176*; cytochrome c is released after collapse of the mitochondrial membrane and triggers apoptosis). Chronic lithium and valproate increase cytoprotective Bcl-2 protein levels in the brain of rats and in cultured neurons *(124,130)*, and treatment of the cells protects them from glutamate-induced excitoxicity. Glutamate can induce excitotoxicity through overstimulation of the NMDA receptor and, consequently increases calcium influx into the cell. Glutamate exposure triggers the release of cytochrome c from the mitochondria into the cytosol. Pretreatment with lithium blocks glutamate-induced cytochrome c release and apoptosis *(124)*.

The effects of lithium on mitochondrial function may also be mediated more directly through its effects on GSK-3. One of the GSK-3 isozymes, β but not α, appears to localize to the mitochondria *(177,178)*. Mitochondrial p53 has been associated with apoptotic conditions *(179–181)*. Lithium and two other inhibitors of GSK-3 prevented camptothecin (a pro-apoptotic stimuli)-induced release of cytochrome c and caspase 3 activation *(182)*, which could partly result from interactions between GSK-3 and p53. Recently, it was reported that Akt (of the PI-3-K pathway) localizes to the mitochondria inner and outer membranes and that stimulation of Akt translocation to the mitochondria induced inhibitory phosphorylation of GSK-3 *(183)*. These data suggest a mechanism whereby cellular signals may directly impact kinases localized to the mitochondria with actions parallel to those of lithium. Recent data also suggest that GSK-3β phosphorylates Bax and promotes its mitochondrial localization during neuronal apoptosis *(184)*.

Clinical Data for Neurotrophic and Neuroprotective Effects of Lithium and Valproate

Although the preclinical data are convincing, there must be caution in relating those findings to the clinical situation in humans (Tables 2 and 3). Interestingly, recent evidence suggests neurotrophic effects of lithium in patients with bipolar disorder. Two longitudinal studies, both conducted over 4 wk, support lithium's neuroprotective properties. Moore and colleagues *(185)* demonstrated that lithium administration at therapeutic doses increased brain *N*-acetyl-aspartate (NAA) concentration. NAA is a putative marker of neuronal function and viability and was quantitatively assessed via magnetic resonance spectroscopy. Another study conducted by the same group *(186)* found a lithium-induced increase in human brain gray matter (as shown with MRI; Fig. 2). These findings provide support for the assertion that chronic lithium increases neuronal viability and function in human brains. These findings also suggest that lithium's long-term beneficial effects may be mediated by neurotrophic/neuroprotective actions.

Two cross-sectional studies support the findings mentioned earlier. One group *(187)* reported that chronic treatment with lithium was associated with an increase in cortical NAA concentration in patients with euthymic bipolar disorder . Sassi et al. *(188)* found increased gray matter volume in lithium-treated patients with bipolar disorder compared to untreated patients (Table 2).

Conclusions: Implications for the Development of New Therapeutics for Bipolar Disorder

Studies to define the targets of mood stabilizers lithium and valproate have focused on

both initial molecular targets and downstream effects on cellular signaling pathways that are shared between multiple drugs (Table 3). Several potential therapies may derive from our current knowledge of the initial targets of mood stabilizers. Strongest among these is the evidence of the effects of lithium on GSK-3 and IMPase: it is likely that these data may eventually lead to trials with alternate inhibitors of these two enzymes.

Although it was proposed more than a decade ago *(43,48)*, inositol depletion (through lithium inhibition of IMPase and IPPase) remains a viable hypothesis for the mechanism of action of lithium. However, no clinically approved inhibitors of either IPPase or IMPase are available; therefore, it remains difficult to test the inositol depletion hypothesis in patients with bipolar disorder. Past industry efforts have attempted to develop a brain-penetrant IMPase inhibitor by attempting to alter the primary substrate of IMPase, inositol monophosphate *(189)*. Compounds with sufficient inhibition were developed but have failed to advance through clinical trials because they are highly charged *(190)* or extremely lipophilic *(191)*, both of which limited the bio-availability in the brain (for review, *see* ref. *189*).

The published crystal structure and modeling studies of IMPase may help to develop novel inhibitors *(192,193)*. Downstream molecules (e.g., PKC) of IMPase signaling and the phosphoinositol pathway may also be relevant targets. For example, preclinical studies define the pivotal role of the PKC signaling pathway in the regulation of neuronal excitability, neurotransmitter release, and long-term synaptic events *(67,194,195)*. The finding of its modulation by lithium and valproate as well as evidence from animal models of mania support the hypothesis that the attenuation of PKC activity may be effective in treating mania (for review, *see* ref. *196*; Fig. 3). These findings led to a single-blind clinical trial investigating possible antimanic properties of the PKC inhibitor tamoxifen *(197)*. Although best known for its anti-estrogenic properties, at high concentrations, tamoxifen is also a potent PKC inhibitor *(198,199)*. Initial results are encouraging: tamoxifen treatment resulted in a significant decrease in manic symptoms rated by the Young Mania Rating Scale, with a more than 50% decrease in the Young Mania Rating Scale score in five of seven patients enrolled in the initial trial *(197)*. Larger double-blind, placebo-controlled studies of tamoxifen are underway.

Alternate inhibitors of PKC are under development. Specifically, inhibition of PKC activity has been advanced as a method to treat diabetic complications, and selective PKC inhibitors are in late-stage clinical trials (for review, *see* refs. *200* and *201*) for this indication. One of the compounds furthest in development is LY333531, ruboxistaurin, a selective PKC-β inhibitor *(201,202)*. Several other groups of PKC inhibitors are under investigation, including rottlerin, indolocarbazoles, PKC412, bisindolylmaleimides, and balanol (reviewed in refs. *202* and *203*). Compounds with properties similar to these may be used as potential medications for the treatment of bipolar disorder.

Potent GSK-3 inhibitors are being actively developed by many pharmaceutical companies. In addition to its possible usefulness in the treatment of bipolar disorder *(81)*, inhibition of GSK-3 has been suggested as a potential therapy for numerous diseases. Diabetes and Alzheimer's disease have received the most attention. Diabetes has drawn interest because GSK-3 phosphorylates and deactivates glycogen synthase (for review, *see* ref. *204*). Alzheimer's disease is a target because of the role GSK-3 plays in both the phosphorylation of tau (for review, *see* refs. *205* and *206*) and the assembly of amyloid-β *(76,207)*. Hyperphosphorylation of tau is associated with the formation of neurofibrillary tangles, whereas accumulation of amyloid-β leads to amyloid plaques. Glycogen synthase kinase-3 inhibitors may also be useful for the treatment of cardiac ischemic injury *(208)*, baldness/alopecia (the Wnt pathway is involved in hair growth; for review, *see* ref. *209*), other neurodegenerative disorders *(86,129)*, and stroke and other neurotraumatic injuries *(117,129,132,210)*.

For these reasons, industry has focused major efforts on the development of selective GSK-3 inhibitors. In 2002, it was reported that more than 45 patents for GSK-3 inhibitors had been approved *(211)*, and the majority of pharmaceutical companies have programs to develop specific small-molecule GSK-3 inhibitors. These inhibitors generally act by inhibiting the ability of ATP to bind to its GSK-3 binding site. Therefore, ATP competitive inhibitors block GSK-3-mediated phosphorylation of all GSK-3 substrates. A more recent development is the synthesis of compounds that inhibit GSK-3 by alternate mechanisms, such as competition for substrate binding sites *(212,213)*. It has been suggested that this class of inhibitors holds the promise of inhibiting GSK-3-mediated phosphorylation of some substrates but not others *(214,215)*. A study also identified the element zinc as an inhibitor of GSK-3β *(216)*. Other preclinical research has identified proteins that naturally inhibit GSK-3 (FRAT1, components of axin), sometimes in a pathway- or substrate-specific manner (for review, *see* ref. *81*). Understanding the exact mechanism by which these proteins inhibit GSK-3 may lead to another class of compounds. Early phase clinical trials (likely for Alzheimer's disease or diabetes) of GSK-3 inhibitors will likely be completed in the near future; it is expected that these compounds will also be tested for efficacy in the treatment of bipolar disorder *(81)*.

Additionally, there is a growing body of data suggesting that agents that directly modulate neurotrophic cascades (such as the CREB/BDNF/bcl-2 cascade) may have use for the treatment of mood disorders (for review, *see* refs. *19* and *217*). Specifically, they may represent a mechanism to attenuate or reverse the deficits in neuroplasticity and cellular resilience observed in patients with bipolar disorder (described earlier in this article). One approach to increase CREB activity, and thus neurotrophic signaling, uses inhibition of phosphodiesterases (PDEs), the enzymes responsible for the breakdown of cAMP. AC-mediated production of cAMP leads to activation of PKA, and subsequent phosphorylation-mediated activation of CREB. In turn, CREB increases transcription of many genes, including BDNF and Bcl-2 (for review, *see* ref. *218*). To support this finding, controlled clinical trials *(219–222)* have demonstrated that rolipram, a specific inhibitor of the high-affinity cAMP PDE4, may have antidepressant efficacy in patients with depression. Although the overall literature regarding the use of rolipram suggests that PDE inhibitors may have antidepressant efficacy and may have a faster onset of action, the potential use of rolipram for depression was limited because of side effects such as nausea and emesis. However, a new generation of PDE inhibitors is in development. In addition to the use of PDE inhibitors, alternative methods to activate neurotrophic signaling pathways are actively being developed for the treatment of classic neurodegenerative disorders and will likely be used to support principle trials in bipolar disorder.

Although not discussed in any great detail in this article, the following systems could be targeted for the development of novel therapeutics in addition to the ones discussed earlier. The antimanic effect of valproate and carbamazepine may be explained by the property of these drugs to act on high-frequency opening of sodium channels. Valproate's direct inhibitory effect on HDAC could lead to changes in gene expression (for an excellent review of HDAC inhibitors in oncology trials, *see* ref. *223*), protein levels, and functional alterations as desired outcome of psychotropic medications. Valproate's enhancement of GABAergic neurotransmission suggests the possible utility of enhancing GABA levels as a therapeutic approach in the treatment of bipolar disorder (for a recent review, *see* ref. *224*). Abnormalities of the hypothalamic–pituitary–adrenal (HPA) axis may play a role in mediating the phenotypic expression of certain affective states *(225,226)*, and this system has been targeted by employing inhibitors of glucocorticoid synthesis *(227,228)*, antagonists of GR *(229,230)*, hydrocortisone to downregulate the HPA axis (as a proof of concept study, *see* ref. *231*), and dehydroepiandrosterone, which is used as a functional antagonist of the actions

of glucocorticoids *(232)*. As the glutamatergic system is a major determinant of neuronal plasticity and is modulated by the HPA axis agents that modulate this system *(233)* may have use in the long-term treatment of severe mood disorders. Generally, glutamatergic modulating strategies include NMDA antagonist, inhibitors of glutamate release agents, metabotropic receptor agonists/antagonists, and AMPA potentiators (for review, *see* ref. *233*).

Therefore, neuroprotective strategies used for the treatment of neurodegenerative disorders may have an important role in the treatment of mood disorders and are currently being tested in "proof-of-concept" studies in patients *(218)*. Such studies may ultimately lead to a better understanding of the pathophysiology of recurrent unipolar depression and bipolar disorder and may result in the development of improved therapeutics. Several strategies are on the horizon to develop new medications for the treatment of bipolar disorder based on both the mechanism of action of existing medications and by targeting drugs toward the underlying pathophysiology of bipolar disorder. Note that although theories abound, ultimate validation (especially considering available animal models of bipolar disorder *[60,234]*) will rest in the results of clinical trials. We hope that the approaches we outlined (and other similar ones) will lead to clinical trials and, ultimately, improved medications for the treatment of those suffering from bipolar disorder.

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